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at the center. Attention is thus called to the difficulty of securing reproducible values of the wavelength for such lines when the spectrograph slit is placed parallel to the axis of the arc, especially if an astigmatic spectrograph is used. With the slit at right angles to the arc at its middle point, on the other hand, it is easy to obtain reproducible results.

Our conclusions may be summarized as follows:

1. It has been shown that the wavelengths of many lines in the iron arc spectrum depend upon the portion of the source used.

2. These variations in wavelength appear not to be due to a general increase in pressure in the vicinty of the negative pole, but the questions of a local increase in pressure and the possible effect of density are still under investigation.

3. The energy distribution in the arc has been shown for two types of lines.

4. Some working conditions whose observance favors the obtaining of reproducible values of wavelength have been quantitatively determined.

AN EXPERIMENTAL STUDY OF LIPOLYTIC ACTIONS

By K. George Falk

HARRIMAN RESEARCH LABORATORY, ROOSEVELT HOSPITAL, NEW YORK

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Purpose of this Investigation. The chemical changes which occur in animal and vegetable growth have focused attention in recent years upon a group of catalytic agents, the enzymes, which are capable of accelerating these changes. The study of the chemical nature and behavior of enzymes is, however, extremely difficult because of the complexity of the substances which occur in living matter and which constitute in most cases the material upon which the enzymes act. Thus most enzymatic reactions involve changes in substances such as proteins or starches, which are themselves of unknown chemical structure. There are, however, some which produce changes in simpler substances. Among these are the lipases, or the ester-hydrolyzing (including the fat-hydrolyzing) enzymes. In this case the composition and structure of the initial and final substances involved in the reaction are definitely known, and the uncertain factors due to the chemical nature of the substance acted upon are eliminated.

This investigation on the action of lipases was therefore undertaken. It has now been in progress for a number of years; and a series of papers describing the experiments in detail have been published in the *Journal of the American Chemical Society* for the years 1912, 1913, and 1914. It is the purpose of this paper to summarize the more important results and conclusions. With reference to these it should be stated that the aim of this investigation has been, not so much to follow the changes which the lipase produces in other substances (for example, by measuring the rate at which it causes the hydrolysis of different esters), as to study the changes in the activity of the lipase itself under various conditions, in the hope of obtaining information with regard to the chemical and physical properties of the substance or substances upon which the lipolytic actions depend.

Preparation of Extracts Containing Two Kinds of Lipase from Castor Beans. Lipases were prepared from both vegetable and animal sources. The most satisfactory and interesting material was found to be husk-free and oil-free castor beans. From these beans two distinct kinds of enzyme were readily extracted and separated from each other. These two preparations differed from each other in their hydrolytic action upon esters. Under certain fixed conditions the one was found to exert a comparatively greater action on ethyl butyrate than on glyceryl triacetate; the other to exert a comparatively greater action on glyceryl triacetate than on ethyl butyrate. The two kinds will be called *esterase* and *lipase*, respectively. Ethyl butyrate was used in these hydrolysis experiments as an example of a simple ester not readily hydrolyzed by water; glyceryl triacetate, as an example of an ester analogous to the naturally occurring fats and oils, from which it differs, however, by its greater solubility in water which makes it more convenient in comparative experimental work.

The esterase of castor beans was found to be associated with substances soluble in water; for clear aqueous solutions of it are obtained by direct extraction with water, dialysis, and filtration. The lipase of castor beans was obtained by extracting the water-insoluble castor-bean preparation with 1.5 normal sodium chloride solution, in which it shows a maximum solubility, and removing the salt by dialysis. In this way a mixture containing the lipase in suspension is formed.

There was no indication of the presence of a co-enzyme with either the esterase or lipase. The identity of the esterase with glycerophosphatase described by Plimmer (*Biochem. J.*, **7**, 43; 1913) was made probable.

Presence of These Lipases in Other Materials. Soy beans were found to contain no esterase, but to contain a lipase having an appreciable solubility in water, but showing again a maximum solubility in a 1.5 normal solution of sodium chloride. Both esterase and lipase were found to be present in human intestinal secretions obtained by means of duodenal tubes. The esterase predominated in the secretions when no food had been taken for some time previously; and it is therefore probably present in the intestinal juice (*succus entericus*). The lipase predominated after the ingestion of food; and it therefore doubtless occurs in the pancreatic juice and bile.

Effect of Neutral Salts on the Rate of Hydrolysis of Esters by Enzymes. The effect of a number of neutral salts on the rate of the hydrolytic actions produced by these enzymes was studied systematically over wide ranges of concentration. Similar results were obtained with the enzymes from different sources. In some cases the added salts showed very marked differences in their effect on the hydrolytic actions of the two enzymes on their respective esters. The esterase action, for instance, was retarded by the presence of sodium chloride or sodium bromide, the retardation being distinct even at a concentration of 0.005 normal and increasing with increasing concentration of the salts. The lipase action, on the other hand, was increased by these salts up to a concentration of 0.1–0.2 normal, and was then decreased, this decrease becoming considerable at high concentrations. Sodium fluoride produced a very strong retardation with both enzymes even in solutions as dilute as 0.1 normal or less. The retarding action of sodium iodide was intermediate between that of the chloride or bromide and the fluoride. Other uni-univalent salts and certain uni-bivalent and bi-bivalent salts were studied similarly. Some of the bivalent radicals or ions, for example sulphate, were found to increase the lipolytic actions.

The possibility that a deleterious action is exercised on digestive processes by bromide and iodide when administered therapeutically in large amounts or over long periods of time was indicated by these results. The inhibiting actions of these salts were shown to be due to the fact that they precipitate or coagulate the enzyme-material. The coagulations were to some extent reversible at first, but long contact with the salt rendered them irreversible.

Effect of Manganese Salts as Oxygen Carriers. Of all the salts studied manganous sulphate produced the greatest accelerating action with castor and soy beans. In all probability this increased action is due to a large extent to the effect of the manganese as an 'oxygen carrier' in

converting inactive material present in the bean into active enzyme. For it was found that when an original castor-bean preparation has been made inactive by heating its solution, it can be partially reactivated by adding manganous salt and passing a stream of air through the solution. The active substance is also produced by placing a solution or suspension in contact with an anode and submitting it to a long-continued electrolysis. These facts evidently support the explanation of the formation of active enzyme from inactive material by oxidation; but hydrolysis is perhaps also a factor.

The cycle dead, living, dead, occurs here in perhaps one of its simplest manifestations, exemplified by the transpositions inactive material of the bean, active enzyme, inactive or 'killed' enzyme material. The possibility of such a regenerative action occurring in the growth and development of the castor bean led to testing the oil-free kernel for manganese. A definite test for it was obtained; and the amount present was estimated to be 0.0006% of the oil-free kernel, or 0.008% of its ash.

Effect of Alcohols and Esters on the Rate of Hydrolysis Caused by Lipases. Methyl and ethyl alcohols were found to exert retarding effects on the rate of hydrolysis—effects which continuously increased with increasing concentration of the alcohols. Methyl alcohol retarded the hydrolyses somewhat more than did ethyl alcohol. Glycerin, on the other hand, had no effect even at a concentration of 25%. The retardation was shown to be due to coagulation of the enzyme.

Since the simple esters are similar in physical properties to the simple alcohols, it was thought probable that they would exert similar coagulating or inactivating actions on the active enzyme. Methyl acetate should then exert greater retarding action on the enzyme than ethyl acetate, while with glyceryl triacetate, the retardation might well be negligible. Similarly, esters containing the lower acid radicals, such as ethyl acetate, might be expected to exert retarding effects; while esters containing the higher radicals such as ethyl butyrate, might have considerably less effect. These hypotheses were tested, and were found to be confirmed. From these results the glycerides of the higher fatty acids which occur in nature would be expected to exert no inhibiting action on the lipase materials.

These actions of the esters on the lipases serve to explain part of the selective actions of the lipases which have been described in the past. They make it evident that the action of the substrate (substance acted upon) on the enzyme must in all cases be taken into account when considering reactions of enzymes.

In the case of the lipase material it was shown that a definite quantity of the enzyme can react with only a definite quantity of glyceryl triacetate in a given time. When the enzyme and ester are present in this ratio an increase in the amount either of ester or of lipase material does not increase the extent of the action.

Preparation, Composition, and Activity of the Solid Lipase-Materials. Solid esterase preparations, active as a rule, were obtained by precipitating the filtered and dialyzed aqueous extracts with acetone. The dialyzed salt-extracts, which contained the lipase material in suspension, give an inactive preparation after filtration, washing of the precipitate, suspension of it in acetone, and standing in this solvent. On the other hand, on standing in water for about two weeks, the soluble esterase-preparation lost its activity, while the insoluble lipase-preparation retained its activity unchanged.

The nitrogen-content of different preparations of esterase, referred to the ash-free and moisture-free substance, ranged from 15.4 to 16.3%, and the phosphorous-content ranged from 0.36 to 0.90%. The ash from these preparations amounted to 5%. Tests made upon them showed the presence of no carbohydrate, of much tryptophane, of much aromatic-group compounds, and of a trace of tyrosine. The solid preparations from the lipase material showed a more constant composition, giving an average nitrogen-content of 16.8%, referred to the ash-free and moisture-free substance, and an average phosphorus-content of 0.68%. The ash was 4.3%. The preparations gave a negative test for carbohydrates, a faintly positive one for tyrosine, a distinctly positive one for tryptophane, and a strongly positive one for aromatic groups. The forms of combination of the nitrogen in the different preparations were found to be the same as those recorded in the literature for typical proteins from various sources, with minor differences in the relative amounts of the various amino acids present. About 25% of the nitrogen was present in the form of arginine, a characteristic of seed proteins. Similar results were found with the soy-bean preparations.

These analyses, taken in connection with the method of preparation which removed all fatty (ether-soluble) substances, show that both esterase and lipase preparations are essentially protein in character. The esterase preparation may be considered to be an albumin, the lipase preparation to be a globulin.

The inactivation of these preparations by water, by salt solutions, or by acetone may be compared with the inactivation of the original castor and soy-bean preparations by heat. The loss in weight of these prepa-

rations in a vacuum desiccator over phosphorus pentoxide was not accompanied by loss in activity; but the same loss in weight by heating at 100–110° was accompanied by a loss in activity of from 50–80%. By drying first and then heating, which caused only a 0.1–0.2% greater loss in weight, the same loss in activity was produced. The loss of activity of the esterase-preparation caused by salts is due to coagulation or precipitation; that of the lipase-preparation caused by treatment with acetone is apparently due to dehydration. Hydrolysis of the enzyme may also play a part under some conditions.

Relation between the Hydrolytic Effects of Lipases and Those of Proteins and Amino-Acids. The hydrolytic actions of lipases are intimately connected with protein material. If these actions are due to proteins, it is probable that only part of the complex protein molecule is directly responsible for them. This possibility was studied by measuring the effects of some aminoacids and peptides in causing the hydrolysis of a number of esters. Some of the results, obtained in part by Dr. M. L. Hamlin, are as follows: Glycine, glutamic acid, and aspartic acid exerted hydrolytic actions on methyl acetate, ethyl acetate, glyceryl triacetate, phenyl acetate, ethyl butyrate, ethyl benzoate, and phenyl benzoate. If these esters be arranged in the order of decreasing amounts of hydrolysis, the order is different in the three cases where the action is caused by water, by glycine alone, and by glutamic or aspartic acids. A comparison of the hydrolytic actions of glycine, alanine, and phenylalanine on the seven esters also indicated certain selective actions. The hydrolysis of methyl acetate and ethyl butyrate by solutions of glycine and acetic acid is less than that by corresponding solutions of acetic acid alone. The hydrolysis of these esters by glycine-hydrochloric acid mixtures was not even approximately proportional to the hydrogen-ion concentrations of the solutions. The dipeptides exerted a comparatively greater action on ethyl butyrate than on methyl acetate, while the dibasic aminoacids showed the reverse actions.

The fact that the different esters are hydrolyzed by aminoacids and peptides is not in itself surprising. In its bearing on the enzyme work its interest lies especially in the selective or specific character of some of the actions, which are apparently independent of the hydrogen-ion concentrations, but dependent upon the structure of the aminoacid or peptide. These effects were, to be sure, small, and the specific character of them was not very pronounced; but the possibility of reproducing such selective actions even in a small degree with simple groupings which themselves may occur in proteins, supports the view that more complex

groupings may produce the greater hydrolytic and highly specific actions observed with the natural lipases. That the simple linking together of animoacids in peptide-union is not sufficient to account for the actions is shown by the fact that peptides exert, if anything, a smaller hydrolytic action than the simple aminoacids.

Conclusion as to the Specific Character of Lipolytic Action. From the investigations briefly summarized in this paper it appears that the specific character of the hydrolytic actions produced by lipases is mainly due to two effects; first, the effect of the substrate on the enzyme in causing its coagulation or precipitation, and second, the effect of the enzyme on the substrate arising from the presence in the former of special groupings which may be similar to those contained in simpler nitrogenous substances which also bring about the hydrolysis of esters.

THE HYDRATION OF THE IONS OF CESIUM CHLORIDE DERIVED FROM TRANSFERENCE EXPERIMENTS IN THE PRESENCE OF RAFFINOSE

By Edward W. Washburn and Earl B. Millard

LABORATORY OF PHYSICAL CHEMISTRY, UNIVERSITY OF ILLINOIS

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The hydration of ions has attracted the attention of chemists for a number of years, and a large amount of evidence has been accumulated to show that ions are hydrated to a greater or less extent. One of the strongest pieces of evidence in favor of this view has been obtained by transference experiments in the presence of a non-electrolyte. If at the end of such an experiment the ratio of water to non-electrolyte has changed in the solutions around the electrodes, either the ions have carried water from one electrode-portion into the other, or they have carried the non-electrolyte in the opposite direction.

In a previous investigation by E. W. Washburn[1] the relative ionic hydrations of the chlorides of lithium, sodium, and potassium in 1.2 molal aqueous solution at 25° were derived by means of transference experiments in the presence of a suitable non-electrolyte as a reference substance. The object of the present investigation was to extend these data so as to include cesium chloride, which seemed desirable since there was much reason to believe that the cesium ion is the least hydrated of all the alkali ions.

The method employed consisted essentially in passing at 25° a measured quantity of electricity through a solution of cesium chloride con-